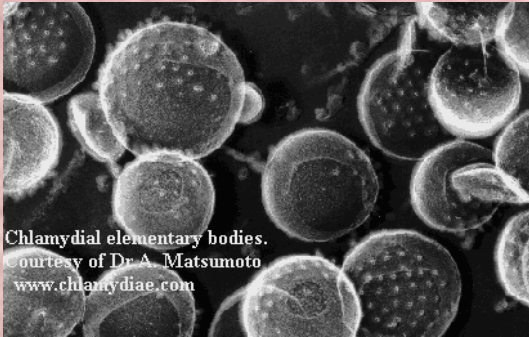


IN THE NAME OF GOD



Chlamydial elementary bodies.  
Courtesy of Dr. A. Matsumoto  
[www.chlamydiae.com](http://www.chlamydiae.com)

**Journal Club & MSc Seminar**

# ***Chlamydia trachomatis vaccine***

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- ✓ Obligate intracellular coccoid parasites
- ✓ contain DNA and RNA, and ribosomes
- ✓ lack ATP, biosynthetic pathways
- ✓ cell wall but peptidoglycan absent -
  - ❖ use disulfide bonds
- ✓ non motile



Chlamydial elementary bodies.  
Courtesy of Dr. A. Matsumoto  
[www.chlamydiae.com](http://www.chlamydiae.com)

✓ **Gram negative**

✓ **Characteristic development cycle**

- **Elementary body (infectious form)**

- **Reticulate body (metabolic form)**

## Chlamydial Morphologies

### ■ **Elementary body**

0.25 - 0.3  $\mu\text{m}$  diameter

electron-dense nucleoid

Released from ruptured infected cells.

Human to human

& bird to human.

### ■ **Reticulate Body**

Intra cytoplasmic form 0.5 - 1.0  $\mu\text{m}$

Replication and growth. ( Inclusion body )

without a dense center.

# Chlamydiaceae Family

## (species that cause disease in humans)

Species (genus)	Disease
<i>C. trachomatis</i> 2 biovars, non-LGV LGV	Trachoma, NGU, MPC, PID, conjunctivitis, Infant pneumonia, LGV
<i>C. pneumoniae</i>	Pharyngitis, bronchitis, pneumonia
<i>C. psittaci</i>	Psittacosis



# *C trachomatis*

Trachoma

conjunctivitis

proctitis

urethritis

salpingitis

Lymphogranuloma venereum

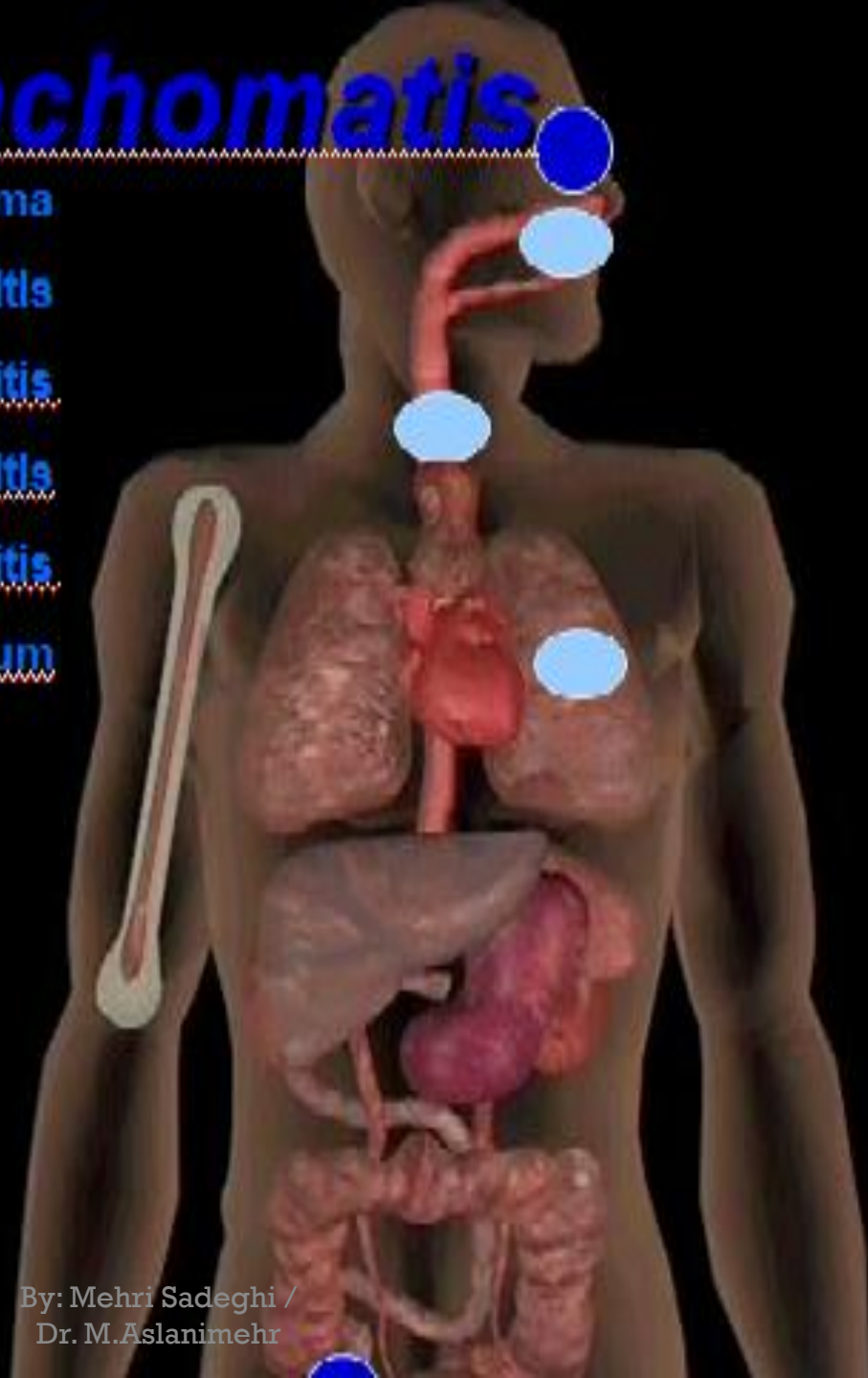
## *C psittaci* & *C pneumoniae*

Upper respiratory infection

Bronchitis

Pneumonia

By: Mehri Sadeghi /  
Dr. M. Aslani-mehr



## ❑ Female Sequelae

Chlamydia can increase HIV transmission 3-5 fold

Up to 15% risk of pelvic inflammatory disease (PID) with untreated chlamydia

PID outcomes:

- Infertility (1 in 5)

- Ectopic pregnancy (1 in 10)

- Chronic pelvic pain (1 in 5)

## □ The value of animal models

animal models are useful and convenient, they must provide data about vaccination that will eventually be transferrable to the human situation. In the case of chlamydial STIs, the mouse model is the most widely used model for infection, pathogenesis and vaccine studies. Primary genital tract infections of female mice with elementary bodies of the mouse-adapted *Chlamydia muridarum* strain are enough to cause tubal dilatation since a consistent observation is the development of hydrosalpinx shortly (1–2 days) after initial chlamydial infection in this model. Hydrosalpinx characteristically is also associated with tubal factor infertility in humans making this model useful in this respect.

➤ However this observation in the murine model contrasts with documented evidence from the guinea pig *Chlamydia caviae* model of a primary genital tract infection in which chronic oviduct pathology was reported in only 12% of the animals, even though almost 80% were infected

❖ the guinea pig model, with observed pathology following primary chlamydial infections and anatomy, and physiology similar to the human female genital tract, more closely resembles human chlamydial disease than their urine.



# Vaccination against *Chlamydia* Genital Infection Utilizing the Murine *C. muridarum* Model

The murine model of *C. muridarum* genital infection has been extremely useful for identification of protective immune responses and in vaccine development. Although a number of immunogenic antigens have been assessed for their ability to induce protection, the majority of studies have utilized the whole organism, the major outer membrane protein (MOMP), or the chlamydial protease-like activity factor (CPAF). These antigens, alone and in combination with a variety of immunostimulatory adjuvants, have induced various levels of protection against infectious challenge, ranging from minimal to nearly sterilizing immunity.

# ➤ Whole Organism Vaccines— First-Generation *C. trachomatis* Vaccines

Initial attempts to develop an effective vaccine for controlling both animal and human chlamydial infections began with the use of inactivated or live, attenuated whole organism preparations in the 1950s. These vaccines can offer a degree of protection but are far from ideal. Common problems are the cost and the complexity of production, the requirement for cold storage, the presence of antigens which can induce autoimmunity or immunopathology, and the limited efficacy in neonates with high levels of maternal antibodies

## ➤ Live Attenuated Organisms

The first vaccines that were used against Chlamydiaceae were **live vaccines**. With this method of immunization, attenuated or modified living chlamydial organisms were used. The development of attenuated strains usually happens by a number of passages of the **wild-type strain in different types of cell cultures or by chemical mutagenesis**. Due to the passages, one or more mutations could arise, resulting in a nonvirulent attenuated strain. **Live attenuated vaccines can elicit humoral and cellular immunity, because they replicate in a manner analogous to the target pathogen, promoting the processing and presentation of antigens in a way that is most similar to the natural infection.**

❖ On the other hand, they can also revert to the virulent wild-type strain **resulting in disease or persistent infection.**

Whole-organism vaccination is unlikely to be attempted in the near future, because there is a risk of immunopathology, the large-scale production of pure chlamydiae is extremely difficult and because of the **possible spread of live Chlamydiaceae in the environment**

❖ In view of the safety aspects (**possible return to the virulent wild type strain**) and the risk for **immunopathological damage**, it seems unlikely that a live attenuated *C. trachomatis* vaccine will be allowed in humans.



## ➤ Inactivated or Killed Organisms

❑ Because live vaccines are not always safe or available, research switched to the use of killed or inactivated organisms. Inactivation was done by heat or chemical treatment.

❑ Their major disadvantage is that they are not able to replicate anymore, which stresses the need to revaccinate and to use adjuvants. Another consequence of their inability to replicate is that they are poor inducers of cell-mediated immunity although they can induce an adequate level of humoral immunity. Because a strong cell-mediated immunity is needed for clearance of chlamydial infections, inactivated or killed organisms seem to be less suitable for vaccine development against Chlamydiaceae

❖ In this study, Peterson et al failed to elicit a protective response to a vaginal *C. trachomatis* infection in mice immunized intranasally and intraperitoneally with  $1 \times 10^6$  UV inactivated inclusion forming units of *C. trachomatis* serovar E.

## ➤ Subunit Vaccines—Second-Generation C. trachomatis Vaccines

✚ Vaccine candidate antigens, or parts of antigens, may be represented as purified proteins, recombinant proteins or as synthetic proteins .

✚ But subunit vaccines have also some disadvantages. Like inactivated vaccines,

✚ they are poor inducers of cell-mediated immunity, which is very important in the defense against chlamydial infections. Further more, the use of adjuvants is being recommended.

## ➤ Purified MOMP and COMC Preparations

vaccine research mainly focused on this protein. Some results were encouraging while others rather disappointing. Pal et al, found that a chlamydial outer membrane complex (COMC) preparation of *C. muridarum* could induce significantly protective immunity in mice against a genital challenge, while purified MOMP preparations could not.

Some years later, the same research group immunized mice with a purified and refolded preparation of the *C. muridarum* MOMP in combination with Freund's adjuvants.



◆ Igietsme and Murdin ,prepared a MOMP-ISCOM vaccine based on MOMP extracted from C. Trachomatis serovar D. This vaccine was able to produce a Th1 antigen-specific immune response, and immunized mice cleared a vaginal infection within one week.

► From these studies, it is clear that some preparations can induce more protection than others. This is probably due to the difference in extraction method which can influence the preservation of conformational MOMP epitopes, necessary for protection. Although vaccination with refolded, purified MOMP preparations have been reasonable successful, the major drawbacks of these vaccines are that they are very expensive and there are problems to grow chlamydia in bulk, which renders these kinds of vaccines commercially non-viable.

## ➤ Recombinant Proteins

@ For chlamydial vaccines, recombinant MOMP (rMOMP) is generally used.

✚ Systemic immunization of mice with rMOMP from *C. trachomatis* could reduce the number of animals developing severe salpingitis but failed to reduce chlamydial colonization of the lower genital tract. Mice, immunized with rMOMP directly into the Peyer's patches (to stimulate mucosal immunity), shed fewer chlamydiae from the vagina, but showed little reduction in oviduct damage. Furthermore, the number of animals developing severe salpingitis could not be reduced. Although in both cases specific IgG and IgA antibody responses could be observed, they could not completely protect the mice.

# Synthetic Peptides

Studies with MOMP peptides and oligopeptide vaccines showed variable results with maximum partial protection. Preliminary studies in mice indicated that intradermal injection of a peptide from a conserved region of the MOMP of *C. trachomatis*, conferred some protection against the development of salpingitis. In contrast to these findings, Su et al. found that parenteral immunization of mice with an alum-adsorbed synthetic oligopeptide of the *C. trachomatis* MOMP, was ineffective in preventing chlamydial genital tract infection although mice produced high levels of antichlamydial serum IgG neutralizing antibodies..

**Therefore, DNA vaccination which induces both humoral and cellular immune responses can be an alternative method to protect animals from chlamydial infections**



## ➤ DNA Vaccines—Third-Generation *C. trachomatis* Vaccines

The first attempt to generate an MOMP-based DNA vaccine against a genital chlamydial challenge was disappointing .

This vaccine encoded the MOMP gene of *C. muridarum*. Only modest immune response was elicited, but no protection could be established against infection or disease.

In 2010 and 2011, Schautteet et al. studied the ability of a DNA vaccine based on *C. trachomatis* MOMP to protect against genital *C. trachomatis* infection in a recently developed pig model .When administering the vaccine to the vaginal mucosa, a cellular immune response was induced which elicited significant protection in pigs. The infection could not be cleared completely [

In 2008, a pORF5 DNA vaccine was evaluated for its protective immunity in a mouse model of genital chlamydial infection. The vaccinated mice displayed significantly reduced bacterial shedding upon chlamydial challenge and an accelerated resolution of the infection.

Furthermore, the immunized mice also exhibited protection against pathological consequences of chlamydial infection. These results demonstrate the potential of the pORF5 DNA vaccine to elicit protective immunity against a genital chlamydial challenge

➤ **Poly(lactic acid)–poly(ethylene glycol) nanoparticles provide sustained delivery of a *Chlamydia trachomatis* recombinant MOMP peptide and potentiate systemic adaptive immune responses in mice**

**Table 1**

*Chlamydia trachomatis* antigens that stimulate host immune responses to genital tract infection.

Chlamydial antigen	Immune response(s) elicited	Reference(s)
Polymorphic membrane protein D ( <b>pmpD</b> )	Genital tract Th1 cells and IgG2a mucosal antibodies	Eko et al. (2011) [123]
Chlamydial type III-secreted effector protein ( <b>Tarp</b> )	Th1 dominant humoral and cellular responses	Wang et al. (2009) [124]
Protein antigens CT823 and CT144	CD8+ T cell, Th1-polarised CD4+ T cell, Th1-skewed antibody responses	Picard et al. (2012) [125]
<b>MOMP</b> -based serovar E DNA vaccine	Serum IgM, IgG and IgA, CD8+ and CD4+ Tcells in spleen and pelvic lymph nodes	Schautteet et al., 2011 [126]
<i>C. trachomatis</i> serovar D strain <b>pgp3</b> gene	Humoral (serum IgG) and mucosal IgA anti-Pgp3 antibodies	Comanducci et al. (1994) [127]
<b>Pgp4</b> gene	Unknown – but the mutant exhibited decreased expression levels of Pgp3, a potential virulence factor amongst others	Song et al. (2013) [55]
TC0052, TC0189, TC0582, TC0660, TC0726, TC0816 and, TC0828	IgG antibodies with both Th1 and Th2 bias	Molina et al. (2010) [128]
<b>MOMP</b> (CT681), <b>HtrA</b> (CT823), <b>OmcB</b> (CT443), <b>TARP</b> (CT456), <b>GroEL</b> (CT110), <b>Lcr-E</b> (CT089), <b>Nqr3</b> (TC0551/CT279), <b>MAC-perforin</b> (TC0431/CT153), <b>IncA</b> (TC0396/CT119), and the hypothetical proteins CT622, TC0284, TC0313, TC0651, TC0890, and TC0106 (CT016, CT043, CT372, CT601, and CT733).	Human serum IgG, IFN- $\gamma$ -producing CD4 <sup>+</sup> T cells	Finco et al. (2011) [129]
<b>DnaK</b> (CT396)	Human CD4+ T cell responses	Coler et al. (2009) [130]
CT043	CD4+ Th1 cells	Meoni et al. (2009) [131]
<b>OmcB</b> (CT443) And also CT004, CT043, CT184, CT509, and CT611, CT082, CT089, CT322, CT396, and CT681, CT110	T cells, B cells or both B and T cells (OmcB)	Follman et al. (2008) [132]
Enolase (CT587)	Human CD4+ T cells	Goodall et al. [133]
Chlamydial protease-like activity factor ( <b>CPAF</b> )	CD4+ T cell, IFN- $\gamma$	Murthy et al. (2006) [134]
<b>NrdB</b>	CD4+ T cells	Barker et al. (2008) [135]
Heat shock protein 60 (cHSP60)	Cervical IgG and IgA antibodies, IFN- $\gamma$	Agrawal et al. (2007) [136]
Outer Membrane Protein 2 (OMP2)	Humoral antibody responses	Portig et al. (2003) [137]



*Thank you*